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(54) FLAVONOID-CONTAINING AGENT FOR SUPPRESSING SYNTHESIS OF PROTEIN OF HSP27 FAMILY

(57)Abstract:

PROBLEM TO BE SOLVED: To provide the above suppressing agent containing a flavonoid as an active component, effective for suppressing the expression of a heat shock protein group having a specific molecular weight and useful for the treatment of cancer related with the protein and disseminated sclerosis relating to the crisis of the cancer.

SOLUTION: This agent for suppressing the synthesis of heat shock proteins having a molecular weight of 16-40kD contains a flavonoid such as quercetin, catechins (e.g. epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin or their isomers), rutin, baicalein or ougonin as an active ingredient. The agent is preferably administered at a daily rate of 1mg to 10g in terms of flavonoid for adult in 1-4 divided doses by oral or parenteral administration.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the synthetic inhibitor of the protein belonging to the heat shock protein group (it is hereafter called HSP27 family) of a before [40kD(s)] from the molecular weight (kD) of 16kilodalton which contains flavonoid as an active principle. By controlling in-house composition of the protein belonging to HSP27 family, especially the synthetic inhibitor of the protein belonging to HSP27 family by this invention can make the physiological condition of patients, such as the illness with which the protein belonging to HSP27 family is considered to participate in the onset, malignant alteration, or the failure of a therapy, for example, cancer, and multiple sclerosis, able to improve effectively, and can treat the aforementioned illness effectively.
[0002]
[Description of the Prior Art] In spite of the advance of a chemotherapy in recent years, a surgical therapy, radiotherapy, immunotherapy, etc., the malignant alteration of cancer is still concerned with the cause of death by cancer directly or indirectly, and conquest of the malignant alteration of cancer has become one of the big technical problems of future cancer treatment. The malignancy of cancer is defined with the fecundity of cancer, infiltrative, metastatic, etc. With classes of primary carcinoma, as for the transition which is one of the phenomena of malignant alteration, a lifting and a cone organ change transition. Transition of cancer is a compound event and begins from growth of a primary tumor, and the infiltration and growth to balking and the circumference organization from a nuclear power plant blow hole of a cancer cell. A tumor vessel rebirth, invasion into the nearby blood vessel of a cancer cell, migration to the remote part by the blood flow, and adhesion and implantation to a vascular endothelial cell, Furthermore, a new tumor vessel was newly born following the infiltration to the outside of the blood vessel, and initiation of growth in a remote part (transition organization), and it consists of the complicated reaction cascade until it results in formation of a visible metastatic focus soon. generally, cancer is comparatively looked like [what has high malignancy], and is divided into the low thing of malignancy. However, a fundamental cure is not established to the high cancer of malignancy, but a patient results in **** in very many cases at last.
[0003] Moreover, the thermotherapy (hyperthermia; hyperthermia) of cancer is an approach which is going to kill a tumor cell alternatively and is going to treat cancer by warming a cancer organization, and the spotlight is captured in recent years. The cancer treatment by the thermotherapy has many advantageous points -- multiplication-effectiveness is acquired -- by using together with that the cell-lethal effectiveness is acquired by 41-45-degree C comparatively mild warming, a radiation, an anticancer agent, etc., when the biological effectiveness of warm temperature is seen. The cure for the cancer by the thermotherapy is tried at almost all each subject in clinical. one [however,] of the troubles of a thermotherapy -- warming -- it is the warm temperature resistance guided to after transience. That is, since a cancer cell becomes warm temperature resistance temporarily by warming which is the 1st time, the killer cell effectiveness by the next warming decreases. Warm temperature resistance is that the cell (or organization) becomes warm temperature resistance transient to the next

warming by carrying out once sublethal warming about a cell (or organization). The present condition is that performing a thermotherapy in most current facilities is limited to 1 - 2 times per week because of warm temperature resistance.

[0004] Moreover, also in the chemotherapy of cancer, while solid cancers which hardly react to a chemotherapy, such as lung cancer and colon cancer, still exist, resistance-ization an anticancer agent loses its effect soon poses a problem also from the cancer reacted to a chemotherapic drug. According to statistics of the United States in 1988, 49% of the cancer diagnosed in one year is the endogenous resistance which shows resistance from the beginning to a chemotherapy, and 47% of a chemotherapy is effective at the beginning, and it considers as the acquired resistance which recurred once a neoplasm vanished. These facts show that one of the most important problems that bar the effectiveness of a chemotherapy over cancer is the resistance over cytotoxic drugs.

[0005] Moreover, multiple sclerosis (multiple sclerosis, MS) is an inflammatory demyelinating disorder which carries out the failure of the central-nerves white matter specifically, and it is the autoimmune disease it is indicated to be that an immune system attacks the myelin sheath which has wrapped the nerve fiber in the onset mechanism. In many cases, in early stages, multiple sclerosis repeats acute hatred and remission, but it comes to take progressive progress after that gradually. As a thing aiming at a symptom improvement of an acute stage, immunosuppresants, such as azathioprine and cyclophosphamide, have been used for adrenocorticotrophin (ACTH) or an adrenocorticosteroid agent again for the purpose of the recurrence prevention in a stage decrementi, or symptom progress prevention of a chronic advance mold. However, many of drugs with which current and a multiple sclerosis patient are medicated are not like [from which the effectiveness was expected], and the thing which is hard to be referred to as enough -- many side effects are also seen by the nonspecific therapy -- is the present condition. Development of the more specific cure for multiple sclerosis is expected.

[0006] a group discovered by the cell on the other hand when a heat shock protein (it is also called heat shock protein;HSP and stress protein) stimulates a cell by a certain stress, for example, heat, heavy metal, drugs, amino acid analogue, or hypoxia (low concentration oxygen) -- it is protein. The heat shock protein exists in the nature universally, and is produced by bacteria, yeast, vegetation, an insect, and the higher animal including Homo sapiens.

[0007] HSP can be divided roughly into 4 of HSP90 family, HSP(s)(for example, HSP of 90kD or 110kD(s) etc.)70 family, HSP(s)(for example, HSP of 70-73kD etc.)60 family, and low-molecular HSP (s) (for example, HSP of 57-68kD etc.) families (for example, HSP of 20kD, 25-28kD, or 47kD(s) etc.) families from the magnitude of molecular weight, although the class is various. In addition, in this specification, the figure indicated to be HSP immediately after that shall show HSP which has specific molecular weight, for example, HSP of molecular weight 27kD shall be called "HSP27." As mentioned above, although many classes exist in HSP, these differ not only in molecular weight but in structure, a function, or a property etc., respectively. the response to stress -- in addition, there is some protein compounded in configuration and a thing like a meeting of a proteinic folding and an unfolding ** protein subunit and proteinic membrane transport for which the indispensable physiological role is played is shown under the normal environment. These functions as a heat shock protein are called a molecular chaperone.

[0008] The manifestation of the protein belonging to HSP27 family has infiltration in lymph node meta, lymph, or a blood vessel, and correlation remarkable between shorter survival rates in a Homo sapiens breast cancer ("J.Natl.Cancer Inst." 83:170-178, 1991). The protein which belongs to HSP27 family also in gastric cancer has a report that it is a negative prognostic factor ("Br.J.Surg.", 78:334-336, 1991). The manifestation level in the primary-carcinoma cell of the protein belonging to HSP27 family Cancer malignancy, Since there is also a report that there are especially a recurrence rate of cancer and forward correlation ("Breast Cancer Res.Treat." 12: 130 and 1988;" Proc.Am.Assoc.Cancer Res.", 30: 252, 1989) By controlling the manifestation of the protein belonging to HSP27 family, it is possible to prevent the malignant alteration of cancer.

[0009] There is a report that the protein belonging to HSP27 family participates in the warm temperature resistance which poses a problem by the thermotherapy of cancer. When a mouse or a hamster cell is

made to introduce and discover Homo sapiens HSP27 gene, the cell of the warm temperature resistance which survives after a heat shock is guided depending on the amount of the protein of HSP27, and increases ("J.Cell.Biol.", 109:7-15, 1989). Moreover, the variant which came to discover HSP27 regularly can acquire heat resistance now by the chinese hamster cell ("J.Cell.Physiol.", 137: 157, 1988). Although it is guided by alpha-B crystallin ** and heat shock processing, the homology of HSP27 and an amino acid sequence is high protein and it is one of the protein belonging to HSP27 family, the cell which carried out the superfluous manifestation of alpha-B KURISUTARIN also acquires the resistance over heat stress ("J.Cell.Biol.", 125:1385-1393, 1994). By controlling the manifestation of the protein belonging to HSP27 family, this suppresses warm temperature resistance and shows possibility of reinforcing the effectiveness of a thermotherapy over cancer. Moreover, since there is also a report that the proteinic manifestation and the drug tolerance belonging to HSP27 family correlate ("Breast Cancer Res.Treat." 23:178 and 1992;" Cancer Res.", 51:5245-5252, 1991), it is also possible by controlling the manifestation of the protein belonging to HSP27 family to suppress drug tolerance and to reinforce the effectiveness of a chemotherapy.

[0010] alpha-B crystallin ****** whose antigen dominant in immunity in multiple sclerosis is protein belonging to HSP27 family -- things are traced ("Nature", 375:739-740, 1995). A manifestation in the nervous tissue of alpha-B crystallin ** and a multiple sclerosis patient is stronger than a manifestation all over a non-person's who become ill organization, and immunogenicity is very high ("Nature", 375:798-801, 1995). It shows Lycium chinense with an epilogue that that these facts serve as a self-antigen by multiple sclerosis controls the manifestation of alpha-B KURISUTARIN in those with alpha-B crystallin ** and the myelin sheath which are one sort of the protein belonging to HSP27 family to the fundamental therapy of multiple sclerosis.

[0011]

[Problem(s) to be Solved by the Invention] this invention persons made the physiological condition of sick patients, such as cancer and multiple sclerosis, improve effectively in view of the above-mentioned situation, and in order to develop the approach of treating the aforementioned illness effectively, they came examination in piles variously about the compound in which synthetic depressant action is shown to the protein belonging to HSP27 family. Consequently, this invention persons found out controlling specifically composition of the protein belonging to HSP27 family in the cell of the organization for which flavonoid shows symptoms also unexpectedly. That is, by prescribing flavonoid for the patient, composition of the protein belonging to HSP27 intracellular family was controlled, therefore it found out that sick therapies, such as cancer and multiple sclerosis, were possible. This invention aims at offering the synthetic inhibitor of the protein belonging to HSP27 family which can treat the illness of cancer, multiple sclerosis, etc. effectively based on such knowledge.

[0012]

[Means for Solving the Problem] Therefore, this invention relates to the synthetic inhibitor of the heat shock protein (namely, protein belonging to HSP27 family) of a before [40kilodalton] from the molecular weight of 16kilodalton characterized by containing flavonoid as an active principle. In this specification, as for "HSP27 family", molecular weight means the heat shock protein group of 16kD-40kD as aforementioned. As protein belonging to HSP27 family, HSP27 (namely, heat shock protein of molecular weight 27kD) [or HSP28 (namely, heat shock protein of molecular weight 28kD)] of mammalian, HSP25 (namely, heat shock protein of molecular weight 25kD) of Tori, HSP26 (namely, heat shock protein of molecular weight 26kD) of yeast, etc. can be mentioned, for example. in addition, generally, since some differences produce proteinic molecular weight by the difference between for example, the determination-of-molecular-weight approach or experiment conditions, in the protein belonging to HSP27 family For example, like [as HSP27 and HSP28 in mammalian], even if molecular weight notations differ What has whether not clear now they are that they are protein of another ** with which amino acid sequences differ, or the same protein with which only molecular weight notations differ on appearance is contained. The protein belonging to HSP27 family is main heat shock proteins in mammalian among the heat shock proteins belonging to the aforementioned low-molecular HSP family, and shows the description which could exceed the living thing kind and was saved. However, unlike

other heat shock proteins, the protein belonging to HSP27 family has different molecular weight for every kind, and is molecular weight 16kD-40kD and very various protein. Moreover, it is one of the protein which is guided by high alpha-B crystallin ********** processing of the homology of HSP27 and an amino acid sequence, and belongs to HSP27 family.

[0013]

[Embodiment of the Invention] Hereafter, this invention is explained to a detail. Especially the flavonoid contained as an active principle of the synthetic inhibitor of this invention is not limited, but well-known flavonoid can be used for it. As flavonoid used in the synthetic inhibitor of this invention, chalcones, flavanones, flavones, flavonols, FURABANO Norians, flavanols (catechins), isoflavone, or anthocyans can be mentioned, for example. Flavonoid can also be used for coincidence combining two or more flavonoid which can also use independently or is different.

[0014] As chalcones, iso OKANIN (Isookanin), iso carthamin (Isocarthamin), An ISOSA ripple pin (Isosalipurpin), ISOBU thorin (Isobutrin), Isoliquiritin (Isoliquiritin), OKANIN (Okanin), A chalcone (Chalcone), carthamin (Carthamin), KOREOPUSHIN (Coreopsin), SUCHIROPUSHIJIN (Stillopsidin), Neo SAKURANIN (Neosakuranin), BUTEIN (Butein), PEJISHIN (Pedicin), PEJISERIN (Pedicellin), a mallein (Marein), run SEORIN (Lanceolin), or run SEORECHIN (Lanceoletin) is illustrated.

[0015] As flavanones, ARUPINECHIN (Alpinetin), isocarthamidin (Isocarthamidin), Iso SAKURANIN (Isosakuranin), iso sakuranetin (Isosakuranetin), Iso PEJISHIN (Isopedicin), eriodictyol (Eriodictyol), Carthamidin (Carthamidin), Cryptostrobin (Cryptostrobin), SAKURANIN (Sakuranin), sakuranetin (Sakuranetin), SARIPURUPIN (Salipurpin), dihydrowogonin (Dihydrowogonin), Silt MINECHIN (Cyrtominetin), stroboscope PININ (Stropopinin), Naringin (Naringin), a naringenin (Naringenin), Neo carthamin (Neocarthamin), a neo hesperidin (Neohesperidin), A PINOSUTORO bottle (Pinostrobin), PINOSEMBURIN (Pinocembrin), A FARURE roll (Farrerol), a butyne (Butin), BUTORIN (Butrin), Hula BANOOKANIN (Flavanookanin), a hula BANOMA lane (Flavanomarein), FURABANORANSEORECHIN (Flavanolanceoletin), A flavanone (Flavanone), PURUNIN (Prunin), a hesperidin (Hesperidin), The hesperetin (Hesperetin), beret KUNJIN (Verecundin), Gay eriodictyol (Homoeriodictyol), PONSHIRIN (Poncirin), mat ISHINORU (Matteucinol), RIKIRICHI genin (Liquiritigenin), or liquiritin (Liquiritin) is illustrated.

[0016] As flavones, a dirt scene (Acaciin), dirt cetin (Acacetin), An apiin (Apiin), apigenin (Apigenin), wogonin (Wogonin), OROKISHIRIN - A (Oroxylin-A), galuteolin (Galuteolin), Chrysin (Chrysin), chestnut SOERI oar (Chrysoeriol), GURUKO luteolin (Glucoluteolin), gene KANIN (Genkwanin), Kos Moshi Inn (Cosmosiin), diosmin (Diosmin), Geos methine (Diosmetin), SUKUTERARIN (Scutellarin), A SUKUTERA lane (Scutellarein), stroboscope chrysin (Strobochrysin), TEKUTO chrysin (Tectochrysin), fricin (Tricin), TORINGIN (Toringin), nobiletin (Nobiletin), Bayh Carin (Baicalin), a BAIKA lane (Baicalein), A flavone (Flavone), a primetin (Primetin), PEKUTORI nari genin (Pectolinarigenin), PEKUTORINARIN (Pectolinarin), PEDARIIN (Pedaliin), PEDARICHIN (Pedalitin), PONKANECHIN (Ponkanetin), Lina Lynn (Linarin), luteolin (Luteolin), ROIHORIN (Rhoifolin), ROTSUSHIN (Lotusin), or ROTOFURABIN (Lotoflavin) is illustrated.

[0017] As flavonols, aza-REACHIN (Azaleatin), an aza-lane (Azalein), Astragalin (Astragalin), loon KURARIN (Avicularin), AFUZERIN (Afzelin), Aya Nin (Ayanin), anchor in (Icariin), Cuttlefish RICHIN (Icaritin), IZARUPININ (Izalpinin), Isoquercitrin (Isoquercitrin), iso rhamnetin (Isorhamnetin), Elian Ching (Erianthin), aura NECHIN (Auranetin), KANUGIN (Kanugin), GARANGIN (Galangin), faucet gin (Karanjin), Gal DENIN (Gardenin), cannabis citrin (Cannabiscitrin), Xanthorhamnin (Xanthorhamnin), chrysoprase spray NECHIN (Chrysosplenetin), KERUSHITSURON (Quercituron), quercitrin (Quercitrin), Quercimeritrin (Quercimeritrin), KERUSETA gitorin (Quercetagitrin), Quercetagetin (Quercetagetin), quercetine (Quercetin), KEYAKININ (Keyakinin), KENFERIDO (Kaempferid), KENFERITORIN (Kaempferitrin), kaempferol (Kaempferol), GOSSHIPI thorin (Gossypitrin), GOSSHIPIN (Gossypin), GOSSHIPECHIN (Gossypetin), SUPIREOSHIDO (Spiraeoside), DACHISU cetin (Datiscetin), TAPUSHIN (Thapsin), Tangeritin (Tangeritin), a tambourine (Tambulin), A tongue bulletin (Tambuletin), TERUNACHIN (Ternatin), Trifolin (Trifolin), NARUSHISSHIN (Narcissin), NORUIKARIIN (Noricariin), NORUIKARICHIN (Noricaritin),

PATSURECHIN (Patuletin), HIBISU citrin (Hibiscitrin), HIBISU cetin (Hibiscetin), HIPERIN (Hyperin), FISECHIN (Fisetin), flavonol (Flavonol), A PERUSHI Chinese quince (Persicarin), HERUBA citrin (Herbacitrin), HERUBA cetin (Herbacetin), MIKERIANIN (Miquelianin), Myricitrin (Myricitrin), myricetin (Myricetin), MERACHIN (Meratin), the Melysin pudding (Melisimplin), Melysin Preki Singh (Melisimplexin), MERITERUNACHIN (Meliternatin), MERITERUNIN (Meliternin), morin (Morin), ram NAJIN (Rhamnazin), Rhamnetin (Rhamnetin), rhamno citrin (Rhamnocitrin), rutin (Rutin), lei note phosphorus (Reynoutrin), ROBININ (Robinin), or ROBINECHIN (Robinetin) is illustrated.

[0018] As FURABANO Norians, Astilbin (Astilbin), Al Pinon (Alpinon), Aroma DIN drine compounds (Aromadendrin), AMPEROPUCHIN (Ampeloptin), Iso en GERICHIN (Isoengelitin), en GERICHIN (Engelitin), Zelkova Norian (Keyakinol), dihydroROBINECHIN (Dihydrorobinetin), Stroboscope van KUSHIN (Strobobanksin), taxi HORIN (Taxifolin), PINOBANKUSHIN (Pinobanksin), FERAMURIN (Phellamurin), FERAMURECHIN (Phellamuretin), or Fustin (Fustin) is illustrated.

[0019] As flavanols (catechins), AFUZEREKIN (Afzelechin), EPIAFUZEREKIN (Epiafzelechin), epicatechin (Epicatechin), Epicatechin gallate (Epicatechin gallate), Epigallocatechin (Epigallocatechin), epigallocatechin gallate (Epigallocatechin gallate), A catechin (Catechin), catechin gallate (Catechin gallate), GAROKATEKIN (Gallocatechin), or GAROKATEKIN gallate (Gallocatechin gallate) is illustrated.

[0020] As isoflavone, isoflavone (Isoflavon), the irigenin (Irigenin), Iridine (Iridin), OSAJIN (Osajin), ONONIN (Ononin), Genistin (Genistin), genistein (Genistein), Sun Taal (Santal), SOHORABIOSHIDO (Sophorabioside), The sophoricoside (Sophoricoside), daizin (Daidzin), A die zein (Daidzein), tectorigenin (Tectorigenin), Theque tolidine (Tectoridin), Biochanin A (Biochanin A) PUSOIDOBA petit genin (Pseudobaptigenin), PUSOIDOBAPUCHISHIN (Pseudobaptisin), PURUNU cetin (Prunusetin), prunetin (Prunetin), POMIFERIN (Pomiferin), or formononetin (Formononetin) is illustrated.

[0021] As anthocyans, AOBANIN (Awobanin), IDEIN (Idaein), IRISHI cyanine (Ilicicyanin), enin (Oenin), Chrysanthemine (Chrysanthemin), GESUNERIN (Gesnerin), A GESUNE lysine (Gesneridin), a keracyanin (Keracyanin), A salvianin (Salvianin), cyanidin (Cyanidin), Cyanine (Cyanin), delphinidin (Delphinidin), Delphinin (Delphinin), delphin (Delphin), A NEGURE theine (Negretein), a violanin (Violanin), Leech SUCHIJIN (Hirsutidin), leech SUCHIN (Hirsutin), The primulin (Primulin), PURUNI cyanine (Prunicyanin), A peonidin (Paeonidin), peonin (Paeonin), A petunidine (Petunidin), PETSUNIN (Petunin), a pelargonidin (Pelargonidin), pelargonin (Pelargonin), a malvidin (Malvidin), or a malvin (Malvin) is illustrated.

[0022] As flavonoid used in the synthetic inhibitor of this invention especially -- desirable -- quercetine -- [-- namely, 2-(3, 4-dihydroxy phenyl)- 3, 5, and 7-trihydroxy-4H-1-benzopyran-4-ON] -- Rutin (namely, quercetine-3-rutinoside), a BAIKA lane () Namely, 5, 6, 7-trihydroxy -2 - Phenyl-4H-1-benzopyran-4-ON, wogonin (5, 7-dihydroxy-8-methoxy -2 - phenyl-4H-1-benzopyran-4-ON), or catechins can be mentioned. As catechins used as an active principle in the synthetic inhibitor of this invention, the (+) catechin, (+) GAROKATEKIN, (+) catechin gallate, (+) GAROKATEKIN gallate, (-) epicatechin, (-) epigallocatechin, (-) epicatechin gallate, and (-) epigallocatechin gallate are desirable. In addition, pure stereoisomers or those mixture can be used as flavonoid used as an active principle in the synthetic inhibitor of this invention.

[0023] the flavonoid contained in the synthetic inhibitor of this invention -- chemosynthesis -- or it can prepare by extracting from a natural product and refining. Or a commercial item may be used. Moreover, although the catechins used as an active principle in the synthetic inhibitor of this invention are mainly known as tea catechins, and it is not limited to this when extracting from a natural product and refining, extracting from tea is desirable.

[0024] As mentioned above, since tea catechins are contained in tea, a tea extract can also be used as an active principle of the HSP27 composition inhibitor of this invention. In this specification, "tea" means remaining as it is, partial fermentation object, or full fermentation object of raw [, such as the brown [Cammellia sinensis, (L) O.Kuntze] entire plant or its part, for example, a leaf, xylem a root, and a

fruit,] or a dry matter, it is independent, or those parts can be combined and used for arbitration. When using tea leaves as extraction feed, there is a thing of various gestalten, for example, the thing of which phase of the usual tea processing process may be used, and a tea green leaf to finishing tea (desiccation tea) can use all of non-fermented tea, such as half-fermentation tea, such as fermentation tea, such as tea, and oolong tea, and green tea, regardless of extent of fermentation.

[0025] A brown crude extract can be used for the tea extract which is the active principle of the synthetic inhibitor by this invention that what is necessary is just to contain the aforementioned tea catechins therefore. This tea crude extract can be obtained by warm water's (preferably boiling water's) extracting tea, or extracting using an organic solvent. for example, ketones, such as low-grade ester, such as lower alcohol, such as methyl alcohol, ethyl alcohol, n-propyl alcohol, isopropyl alcohol, or butyl alcohol, methyl acetate, ethyl acetate, propyl acetate, or butyl acetate, an acetone, or methyl isobutyl ketone, can be used, and independent [in these organic solvents] as an organic solvent, -- or it can combine suitably and can use by the moisture state further anhydrous or preferably.

[0026] It is desirable to carry out heating reflux at the temperature below the boiling point, being able to use the approach used for the usual crude drug extract as the approach of a water extract and an organic solvent extract, for example, stirring to the tea-leaves (desiccation) 1 weight section using water or an organic solvent 5 - 20 weight sections. An extract process can usually shorten extract time amount for 5 minutes - seven days by carrying out preferably for 10 minutes to 24 hours, and adding supplementary means, such as stirring, if needed. Water or an organic solvent extract can separate insoluble matter by suitable approaches, such as filtration or centrifugal separation. These extracts other than the hot water extract by the conventional method or an organic solvent extract are included in the tea extract which can also use the product processed further as an active principle of the synthetic inhibitor of this invention with various organic solvents or an adsorbent. These tea extracts meet the need, and it condenses and dries, and it can carry out disintegration, or they can be further crystallized and refined from cold water.

[0027] In this way, the obtained tea extract contains as mixture the impurity which originates in raw material tea at coincidence including the catechins (for example, a catechin, epicatechin, GAROKATEKIN, epigallocatechin, catechin gallate, epicatechin gallate, epigallocatechin gallate, or GAROKATEKIN gallate), i.e., tea catechins, contained in tea (especially tea leaves).

[0028] the usual support in which the synthetic inhibitor of this invention is independent [its], or can permit aforementioned flavonoid or aforementioned tea extracts, such as quercetine, rutin, a BAIKA lane, wogonin, or catechins, galenical pharmacy-wise [it is desirable and] or in veterinary medicine -- an animal -- mammalian (especially Homo sapiens) can be medicated preferably. Especially as an administration pharmaceutical form, there is no limitation, for example, parenteral agents, such as oral agents, such as powder, a fine grain agent, a granule, a tablet, a capsule, suspension, an emulsion agent, syrups, extracts, or a pill, or injections, liquids for external use, an ointment, suppositories, a cream of partial administration, or eye drops, can be mentioned. These oral agents For example, gelatin, sodium alginate, starch, Corn starch, white soft sugar, a lactose, grape sugar, mannite, a carboxymethyl cellulose, A dextrin, a polyvinyl pyrrolidone, crystalline cellulose, a soybean lecithin, Cane sugar, fatty acid ester, talc, magnesium stearate, a polyethylene glycol, Excipients, such as a magnesium silicate, a silicic acid anhydride, or synthetic aluminum silicate, According to a conventional method, it can manufacture using a binder, disintegrator, a surfactant, lubricant, a fluid accelerator, a diluent, a preservative, a coloring agent, perfume, corrigent, a stabilizing agent, a moisturizer, antiseptics, an antioxidant, etc. For example, it is the capsule mixed and filled up with the catechin 1 weight section and the lactose 99 weight section.

[0029] As the parenteral administration approach, injection (inside of hypodermically and a vein etc.) or rectum administration is illustrated. In these, injections are used most suitably. For example, in preparation of injections, isotonizing agents, such as nonaqueous solubility solvents, such as water soluble solvents, such as a physiological saline and Ringer's solution, vegetable oil, and fatty acid ester, grape sugar, and a sodium chloride, a solubilizing agent, a stabilizing agent, antiseptics, a suspending agent, an emulsifier, etc. can be used for arbitration other than the flavonoid as an active principle. If an

example is shown concretely, after dissolving (+) catechin 10mg and mannitol 50mg in distilled water, being referred to as 10ml and disinfecting with a conventional method, 2ml is poured distributively to each pint bottle for injection, or it freeze-dries as it is, and considers as injections. On the occasion of use, it dilutes with a physiological saline and considers as a parenteral solution. Moreover, the synthetic inhibitor of this invention may be prescribed for the patient using the technique of a sustained release drug in which the controlled-release polymer etc. was used. For example, the pellet of an ethylene vinyl acetic-acid polymer can be made to be able to incorporate the synthetic inhibitor of this invention, and it can transplant surgically during the organization which should treat this pellet.

[0030] Although the synthetic inhibitor of this invention is not limited to this, it can contain preferably the salt permitted on flavonoid or its physic in 0.1 - 80% of the weight of an amount 0.01 to 99% of the weight. Moreover, the synthetic inhibitor of this invention which contains a tea extract as an active principle can be suitably adjusted so that the flavonoid contained in it may become the aforementioned amount range, and it can be prepared. In addition, it is desirable to pharmaceutical-preparation-ize it using support permissible in galenical pharmacy, in considering the synthetic inhibitor which contains a tea extract as an active principle as the pharmaceutical preparation for internal use. Although the dose in the case of using the synthetic inhibitor of this invention changes with a sick class, a patient's age, extent of a symptom, medication methods, etc. and there is especially no limit, about 1mg-10g is usually prescribed [as an amount of flavonoid] for the patient in taking orally or parenterally in about 1 - 4 steps per day per adult. Furthermore, it is also possible for an application not to be limited to drugs, either and to give it in the form of ingesta as various applications, for example, functional food, and health food.

[0031] In addition, among the flavonoid used for the synthetic inhibitor of this invention, in mouse internal use, the acute toxicity (fifty percent lethal dose) of quercetine is 160 mg/kg (a THE Merck index, the 11st edition, Merck Co., 1278 pages), and, in the case of a mouse intravenous injection, the acute toxicity (fifty percent lethal dose) of rutin is 950 mg/kg (a THE Merck index, the 11st edition, Merck Co., 1319 pages). Moreover, especially toxicity was not accepted in the tea catechins used for the synthetic inhibitor of this invention.

[0032]

[Function] As described above, since the flavonoid contained in the synthetic inhibitor of this invention has the operation which controls specifically composition of the protein belonging to HSP27 intracellular family, if said flavonoid is prescribed for the patient, the biosynthesis of the protein belonging to HSP27 family in a cell will decrease specifically. Therefore, the protein belonging to enhancement of the effectiveness of the cancer thermotherapy relevant to warm temperature resistance with the protein acting as [resistance] the failure to the therapy with which the protein belonging to for example, HSP27 family belongs to prevention and the therapy of the cancer relevant to the malignant alteration, and HSP27 family, or HSP27 family can use said flavonoid for prevention, therapies, etc. of an autoimmune disease relevant to the onset, such as multiple sclerosis. Moreover, since there is also a report that the proteinic manifestation and the drug tolerance belonging to HSP27 family correlate ("Breast Cancer Res.Treat." 23: 178 and 1992;" Cancer Res.", 51:5245-5252, 1991), it is also possible by controlling the manifestation of the protein belonging to HSP27 family to suppress drug tolerance and to reinforce the effectiveness of a chemotherapy.

[0033]

[Example] Hereafter, although an example explains this invention concretely, these do not limit the range of this invention.

Example 1: The various Homo sapiens culture cancer cells below culture of the measurement (1) Homo-sapiens culture cancer cell of the amount of HSP manifestations of a Homo sapiens culture cancer cell were cultivated at 37 degrees C under 5% carbon-dioxide conditions except the time of heat shock processing. The lung cancer cell strain H69 (ATCC HTB 119), the gastric cancer cell strain KATO III (ATCC HTB 103), the colon cancer cell strain COLO205 (ATCC CCL 222), the cancer-cells-of-breast-carcinoma stock MCF 7 (ATCC HTB22), and prostate gland cancer cell stock DU 145 (ATCC HTB 81) was cultivated in the RPMI1640 culture medium which contains inactivation fetal calf serum (it is

hereafter called FBS for short) 10%. 10-8Mbeta-estradiol was added to the culture medium of MCF7. Uterine cancer cell strain HeLa S3 (ATCC CCL 2.2) and the renal cancer cell strain ACHN (ATCC CRL 1611) were cultivated in the MEM culture medium which includes Inactivation FBS 10%. Nerve tumor cell stock (neuroblastoma) SK-N-MC (ATCC HTB 10) was cultivated in the MEM culture medium which includes Inactivation FBS in a nonessential-amino-acid (L-alanine 8.9 mg/l, L-asparagine and H2 O15.0 mg/l, L-asparatic acid 13.3 mg/l, L-glutamic acid 14.7 mg/l, glycine 7.5 mg/l, L-proline 11.5 mg/l, and L-serine 10.5 mg/l) list 10%.

[0034] (2) In the culture medium of said various culture Homo sapiens cancer cells two days after flavonoid processing and heat shock processing seeding, any one of the following flavonoid was added and it cultivated for 24 hours. the concentration in the inside of the culture medium after addition of the used flavonoid -- quercetine (Nakarai Tesuku, catalog number 298-12) 100microM, rutin (Nakarai Tesuku, catalog number 303-19) 100microM, and catechin [(+)-Catechin; Funakoshi [ ] -- Code No.0952 : They were the product made from EXTRASYNTHESE, France]100microM, BAIKA lane (Matsuura medicine business) 20microM, and wogonin (Matsuura medicine business) 20microM. Then, after carrying out heat shock processing for 15 minutes at 45 degrees C, it cultivated at 37 degrees C all night. The control test was carried out like the above except not adding flavonoid.

[0035] (3) It homogenized by the approach of showing below each cell processed for the measurement preceding clause (2) of the amount of HSP manifestations in a Homo sapiens culture cancer cell, and the amount of HSP manifestations was measured in the Western blot technique. The cell processed for the preceding clause (2) Namely, below phosphate-buffered-saline [presentation:KCl=0.2 g/l, KH2 PO4 =0.2 g/l, NaCl=8 g/l, and Na2HPO4 =(anhydrous) 1.15 g/l; Rye cis- buffer (lysis buffer) [1.0%NP-40, 0.15M sodium chloride after washing by] called PBS (-), 50mM tris - HCl (pH8.0), 5 mM-EDTA, 2 mM-N-ethylmaleimide, 2mM phenylmethyl sulfonyl fluoride, 2microg [/ml] leupeptin, and 2microg/ml pepstatin]1ml were added, and it put for 20 minutes in Hikami. Then, 12000rpm performed centrifugal for 20 minutes at 4 degrees C. 10micro of supernatant liquid l after centrifugal was added to PBS(-) 790microl, and 200micro (Dye Reagent Concentrate : Bio-Rad, catalog number 500-0006) of protein assay stain solutions l was added further. For 5 minutes, after putting at a room temperature, the absorbance was measured by 595nm and the protein quantum was performed.

[0036] The SDS polyacrylamide gel electrophoresis of rye SETO which contains equivalent protein by the buffer system (Laemmli and N.K., "Nature", 283:pp.249-256, 1970) of Laemmli was performed using the sample which performed the protein quantum. Blocking following blotting and it was performed after electrophoresis. That is, using protein imprint equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad, catalog number 170-3946), at the room temperature, gel was stuck to 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), and blotting was performed 100V for 3 hours. The buffer which prepared methyl alcohol in addition to the tris glycine buffer (Tris Gly Running and Blotting Buffer;Enprotech, U.S. Massachusetts, catalog number SA100034) which consisted of 0.025M tris and 0.192M glycine, and was adjusted to pH8.5 as a blotting buffer so that it might become at 20% was used. After blotting, the nitrocellulose membrane was incubated for 30 minutes at the room temperature in the 10% skim milk (Snow Brand Milk Products)-PBS (-) solution, and nonspecific association was blocked.

[0037] The anti-Homo sapiens HSP27 mouse monoclonal antibody (StressGen, Victoria, B.C., Canada, catalog number SPA-800) performed primary antibody reactions on the nitrocellulose membrane after blocking. This anti-Homo sapiens HSP27 mouse monoclonal antibody It is the antibody which produced as immunogen HSP24 isolated from the Homo sapiens cancer-cells-of-breast-carcinoma stock MCF 7 (ATCC HTB 22) ("Cancer Res.", 42, 4256-4258, and 1982). It reacts specifically with HSP27 (Homo sapiens HSP27, a chimpanzee HSP27, and sheep HSP27) (" 42, 4256-4258, Cancer Res."1982;" CancerRes.", 43, 4297-4301, 1983). HSP24 and HSP28 react specifically. After primary antibody reactions, every 5-minute room and the solution were exchanged by PBS (-), the slow locking shaker performed two washing, every 15-minute room and the solution were further exchanged with Tween20 (Bio-Rad, catalog number 170-6531) solution PBS(-) -0.1%, and four washing was performed. Finally, every 5-minute room and two washing were performed by PBS (-).

[0038] Secondary antibody reactions were performed after washing termination for 2 hours using 5ml of antibody solutions which diluted and prepared the peroxidase-labeling goat anti-mouse IgG antibody (CAPPEL, catalog number 55550) 5000 times with the PBS (-) solution which contains skim milk 2%. after reaction termination, about the nitrocellulose membrane, the every solution during 5 minutes was changed with the PBS (-) solution, twice, the every solution during 15 minutes was further changed with Tween20 solution PBS(-) -0.1%, and the slow locking shaker performed five washing. the last -- an PBS (-) solution -- every [a for / 5 minutes] -- two washing was performed. After having sprinkled the Western-blotting detection reagent (ECL Western blotting detectionreagent;Amersham, catalog number RPN2106) on the nitrocellulose membrane after removing an excessive PBS (-) solution, and incubating for 1 minute, the excessive detection reagent was removed, a nitrocellulose membrane is stuck to a lap, the package and the reaction side were stuck to the X-ray film (KODAKKU X-OMAT, AR, catalog number 165 1454), it exposed, negatives were developed, and existence of HSP27 was examined. A result is shown in Table 1. Front Naka and "**" mean that the amount of HSP27 manifestations decreased compared with contrast.

[0039]

[Table 1]

Cancer type Cancer cell Quercetine Rutin Catechin BAIKA lane Wogonin uterus HeLa S3 ** ** ** lungs H69 ** stomach KATO III ** ** ** large intestine COLO 205 ** ** kidney ACHN ** ** prostate gland DU 145 ** ** ** milk MCF7 ** nerve SK-N-MC ** ** [0040] The band of molecular-weight abbreviation 27kD was detected one by the control test, i.e., the cell which did not add flavonoid. In addition, association with said anti-Homo sapiens HSP27 mouse monoclonal antibody and a molecular weight marker (soybean typsin inhibitor and cow carbo nick anhydrase) determined molecular weight. Quercetine is the uterine cancer cell strain HeLa as shown in Table 1. S3, the gastric cancer cell strain KATO III, colon cancer cell strain COLO 205, the renal cancer cell strain ACHN, prostate gland cancer cell stock DU The manifestation of HSP27 was controlled in 145 and the cancer-cells-of-breast-carcinoma stock MCF 7. Moreover, rutin controlled the manifestation of HSP27 in the gastric cancer cell strain KATO III, the renal cancer cell strain ACHN, and nerve tumor cell stock SK-N-MC. A catechin is the uterine cancer cell strain HeLa. The manifestation of HSP27 was controlled in S3 and nerve tumor cell stock SK-N-MC. A BAIKA lane is the colon cancer cell strain COLO. 205 and prostate gland cancer cell stock DU The manifestation of HSP27 was controlled in 145. Wogonin is the uterine cancer cell strain HeLa. S3, the lung cancer cell strain H69, the gastric cancer cell strain KATOIII, and prostate gland cancer cell stock DU The manifestation of HSP27 was controlled in 145. That is, a catechin, quercetine, rutin, a BAIKA lane, and wogonin can be concluded to be what has the activity of the synthetic inhibitor which controls the manifestation of HSP27.

[0041]

[Effect of the Invention] As explained in full detail above, flavonoid has the activity of the synthetic inhibitor which controls the manifestation of the protein belonging to HSP27 intracellular family. Therefore, by prescribing flavonoid for the patient, the protein with which the protein belonging to for example, HSP27 family belongs to the cancer relevant to reduction of the effectiveness of the malignant alteration and thermotherapy or HSP27 family can make the physiological condition of the patient of autoimmune diseases, such as multiple sclerosis relevant to the onset, able to improve effectively, and can treat said illness effectively. Moreover, since there is also a report that the proteinic manifestation and the drug tolerance belonging to HSP27 family correlate ("Breast Cancer Res.Treat." 23: 178 and 1992;" Cancer Res.", 51:5245-5252, 1991), it is also possible by controlling the manifestation of the protein belonging to HSP27 family to suppress drug tolerance and to reinforce the effectiveness of a chemotherapy.

[Translation done.]